

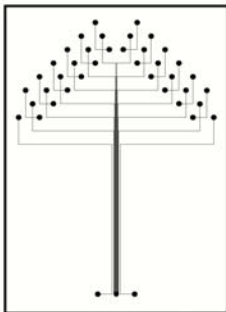
ADVANCES IN MICROFABRICATED CAPILLARY ARRAY ELECTROPHORESIS BIOANALYZERS

Chemistry Department and
Center for Analytical Biotechnology
University of California at Berkeley

1. Microfabricated Capillary Array Electrophoresis "chips" for high throughput genetic analysis - The Next Generation sequencing and genotyping platform.
2. Polymorphism Ratio Sequencing - A clever μ CAE resequencing method.
3. Development of an ultra-high throughput 384 lane μ CAE device.
4. Development of nanoliter sample handling and PCR reactor for integrated low-volume sample preparation and for point-of-analysis devices.

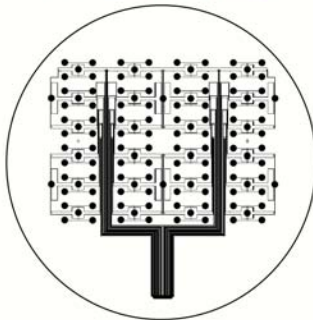
Evolution of the CAE Microplate

12 Channel, 12 Sample



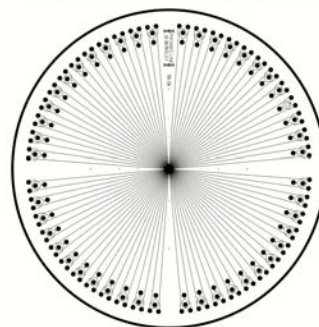
Woolley et al. 1997

48 Channel, 96 Sample



Simpson et al. 1998

96 Channel, 96 Sample (4")

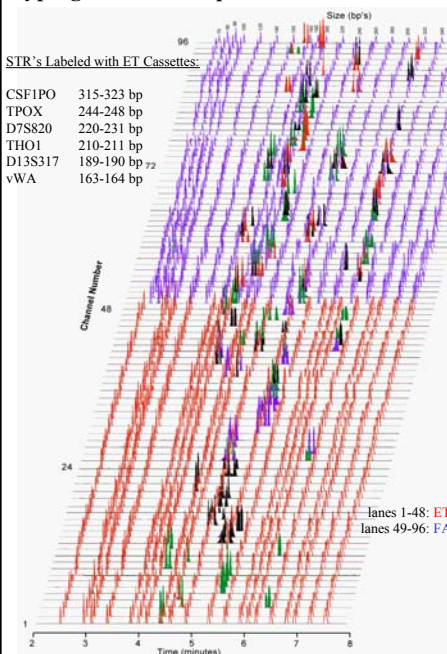


Shi et al. 1999

The true power of microfabrication lies in the production of high-density, high-throughput microfluidic analysis devices

Typing 96 STR Samples in <8 Minutes

Hereditary HaemoChromatosis SNP Genotyping



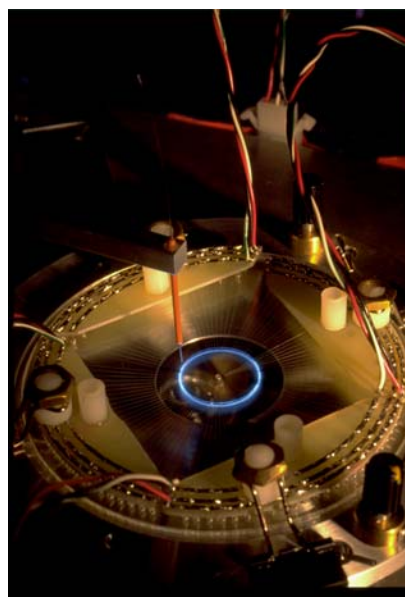
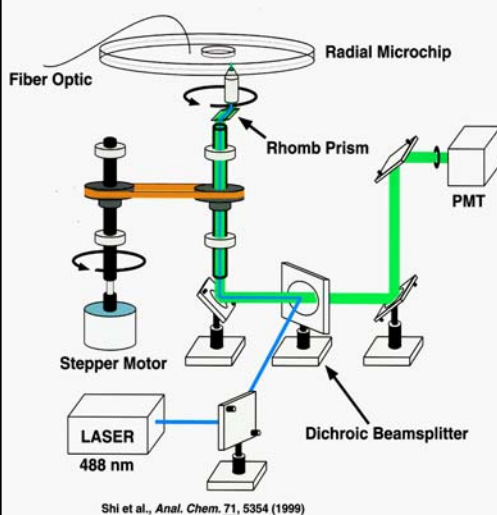
Medintz et al., Clinical Chemistry 47, 1614 (2001)



Medintz et al., Genome Research 11, 413 (2001)

Berkeley Rotary Confocal Fluorescence Scanner

Scanning Fluorescence Microscopy Instrumentation



UC Berkeley

HIGH-THROUGHPUT DNA SEQUENCING ON μ CAE DEVICES

- Layout 96 CE channels ~15-cm long on a 150-mm wafer

Radial CAE Design - Shi *et al.* Anal. Chem. (1999) 71: 5354-61.

Serpentine Channel Geometry - Paegel *et al.*, (2002) PNAS 99: 574-79.

Hyper-Turns - Paegel *et al.*, Anal. Chem. (2000) 72: 3030-37.

- Load high-viscosity gel ($\eta \simeq 50,000$ cp)

High-Pressure Gel Loader - Scherer *et al.*, Biotechniques (2001) 31: 1150-56.

- Eliminate rapid (~3 min) buffer evaporation @ 60° C

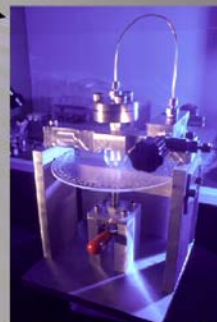
Formamide Sample Loading Buffer

Expanded Buffer Reservoir

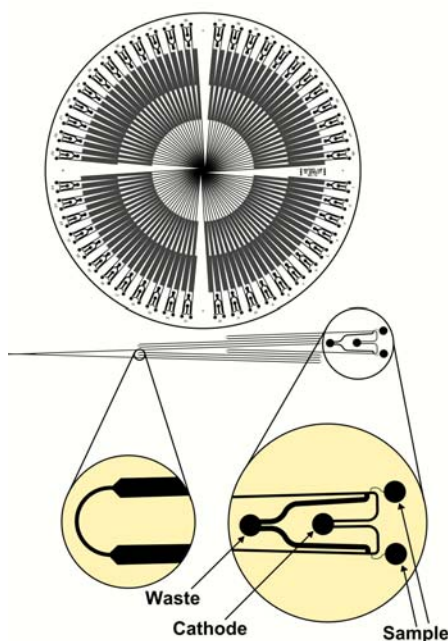
- Electrolysis in reservoirs exceeds buffering capacity

Expanded Buffer Reservoir

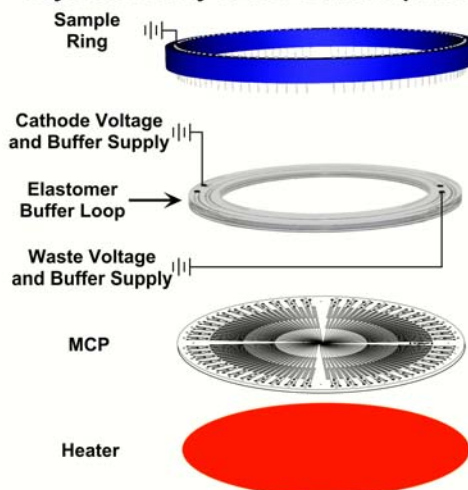
5X TTE Buffer



SEQUENCING PROCESSOR DESIGN

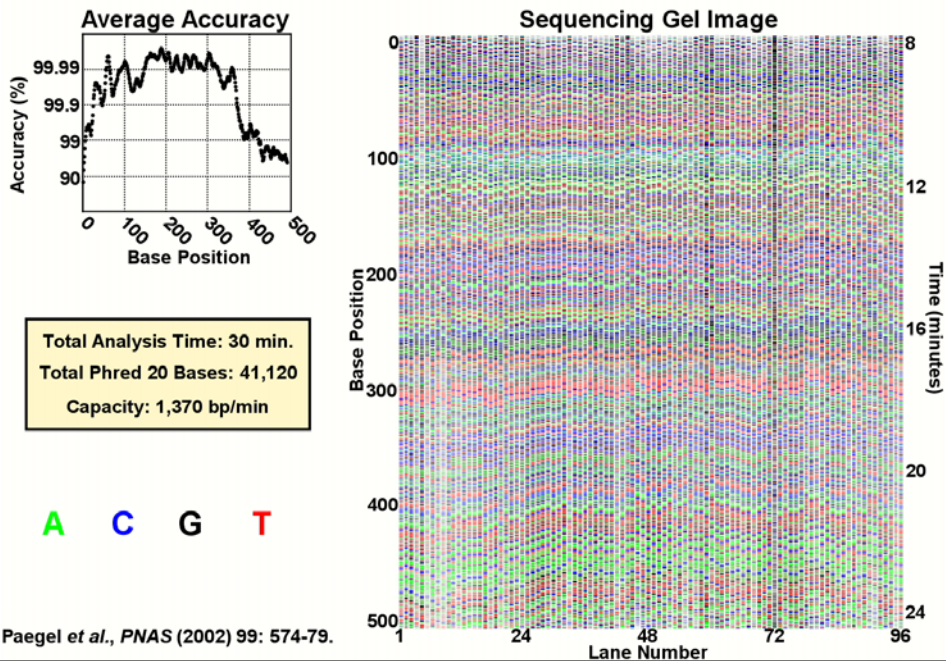


Layer Assembly of MCP DNA Sequencer



Buffer Loop Capacity: 3 mL
Total System Current: 3 mA
Heater Temperature: 60° C

HIGH-THROUGHPUT MCP SEQUENCING of M13mp18 DNA

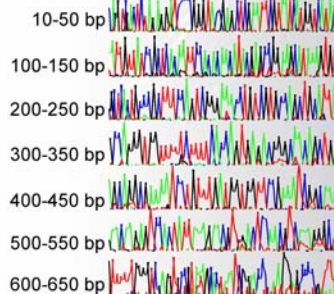


μ CAE Sequencing Optimization: M13 Standards and JGI Production Samples

Commercial Matrix \rightarrow Mixed High & Low M_w LPA
Lower Field \rightarrow 150 V/cm

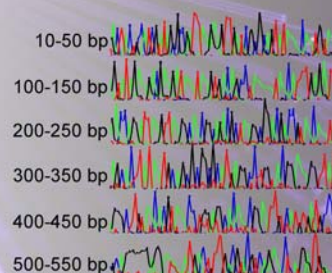
M13 4-Color Standard

Dye-Primer Chemistry
Phred20 Read = 670 bp
Analysis time = 30 min



JGI Production Samples

Dye-Terminator Chemistry
Phred20 Read = 505 bp
Analysis time = 25 min



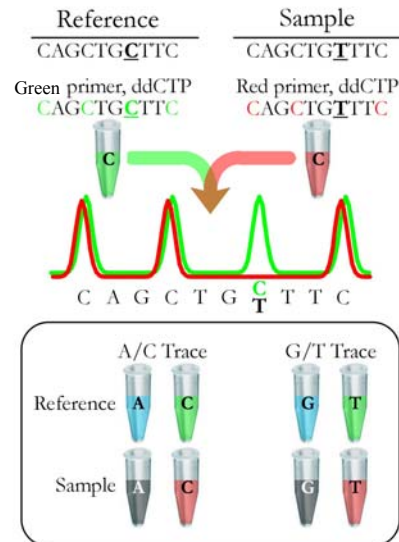
Polymorphism Ratio Sequencing A New Approach for SNP Genotyping

- **Existing Methods**

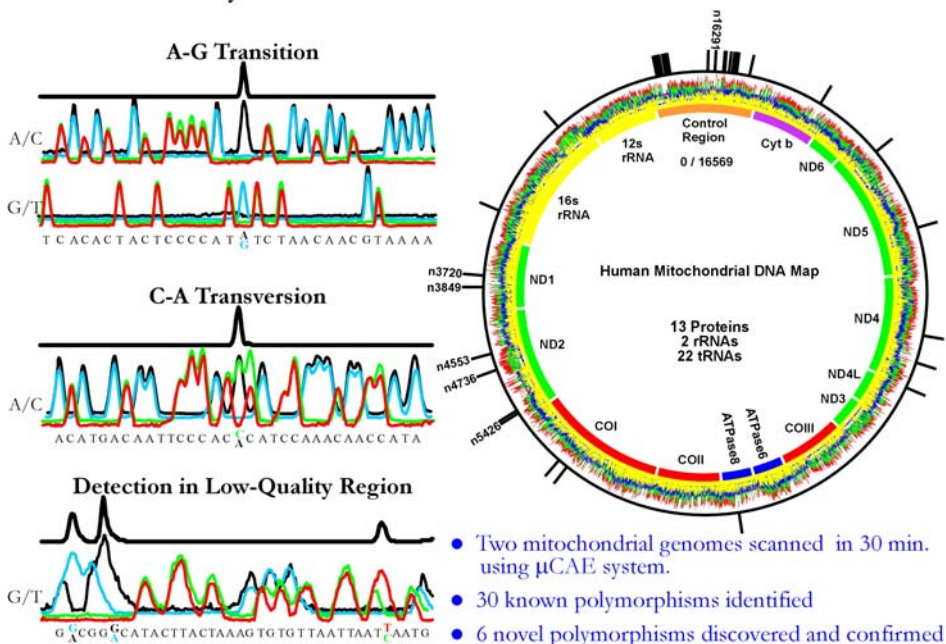
- Microarrays
- Sequencing
- Mini-sequencing
- Allele-specific PCR
- OLA
- SSCP
- Pyrosequencing

- **Polymorphism Ratio Sequencing**

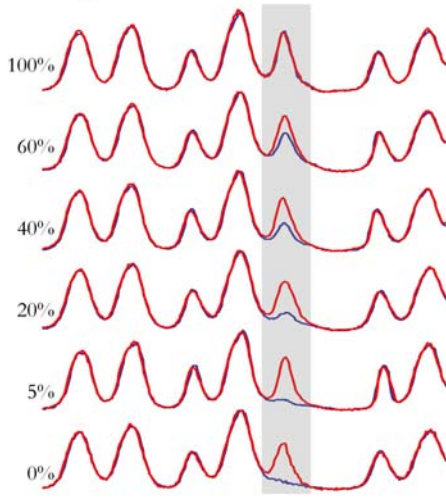
- SNP discovery and screening
- Focus on sequence changes
- Unambiguous SNP detection
- Extended read-lengths
- Quantitative pooled analysis



PRS Analysis of the Human Mitochondrial Genome

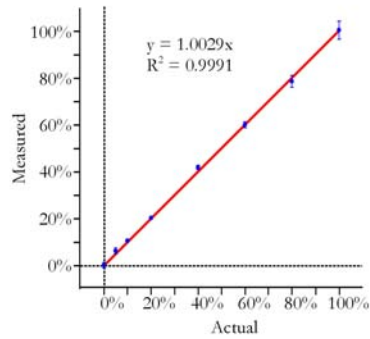


Quantitative Pooled Allele Frequency Determination



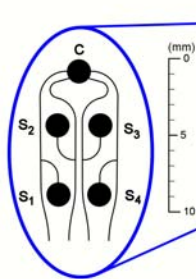
CCAGCCAGGCAACCTTCTAGGTACGACC

- 5% minor allele frequency LOD
- Single base resolution not required



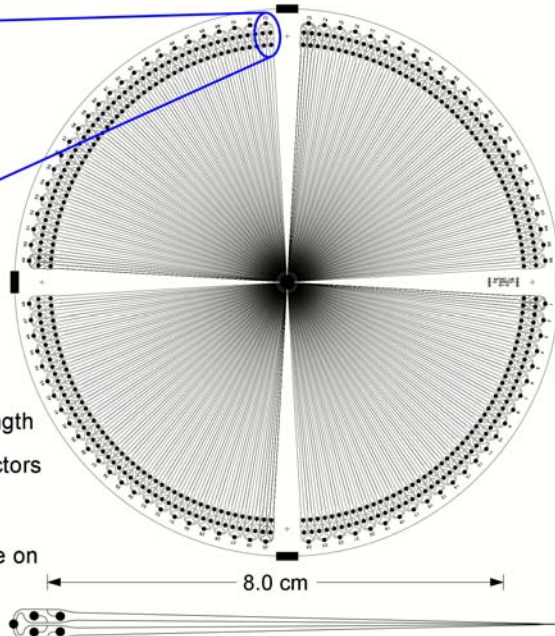
- **PRS is useful for:**
 - Population allele frequency determination
 - High-throughput association studies using patient pools
 - Rare variant detection

Design of the 384-Channel CAE Microdevice



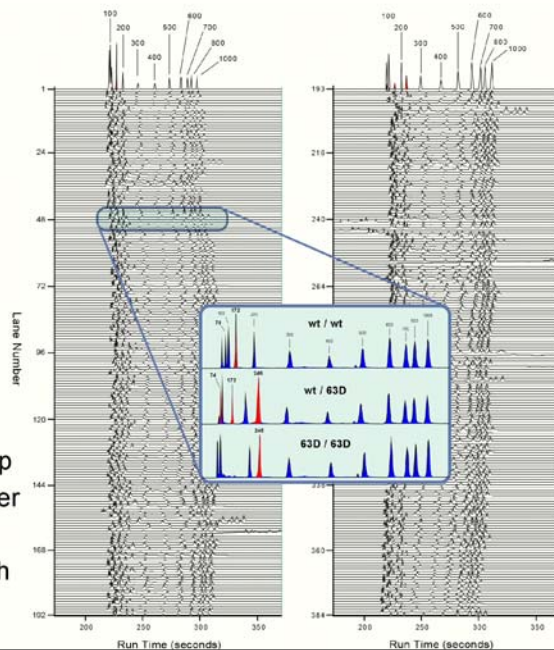
C = CATHODE WELL
S_n = SAMPLE WELL

- 384 parallel channels
- 8.0 cm effective separation length
- Fluidically-balanced direct injectors
- 2.5 μ L sample volume
- Highest device density possible on a 200-mm dia. wafer

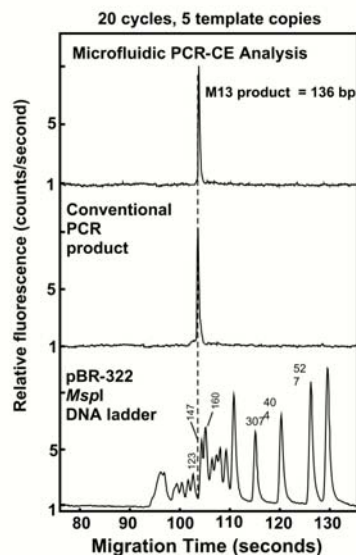
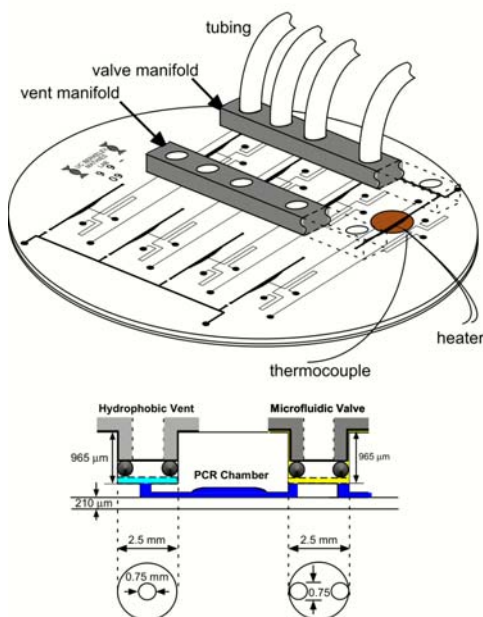


Genotyping 384 Samples for the H63D Mutation in *HFE*

- 384 Samples run in parallel
- 350 sec. analysis time
- 98.5% accuracy
- H63D mutation in hemochromatosis detected by PCR-RFLP
- 246 (mt) *Mbol* → 172/74 (wt) bp fragments with a 100 bp ladder
- Sieving matrix: 5% pDMA with 1 μ M thiazole orange



Integrated Genomic Analysis Device



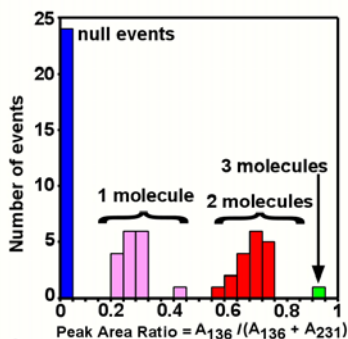
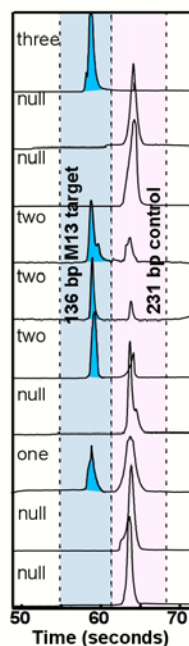
30 seconds/cycle

Temperature ramp rates to 10 °C

Direct injection and separation on 5-cm CE channel

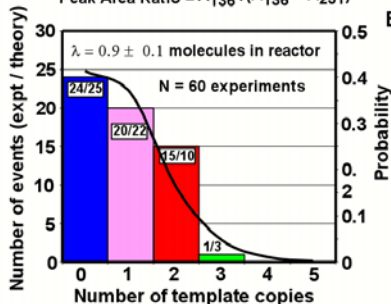
Lagally *et al.*, Sens. Actuators B 63, 138-146

Stochastic Amplification from Single Template Molecules



~ 1 M13 molecule present in chamber at start of amplification

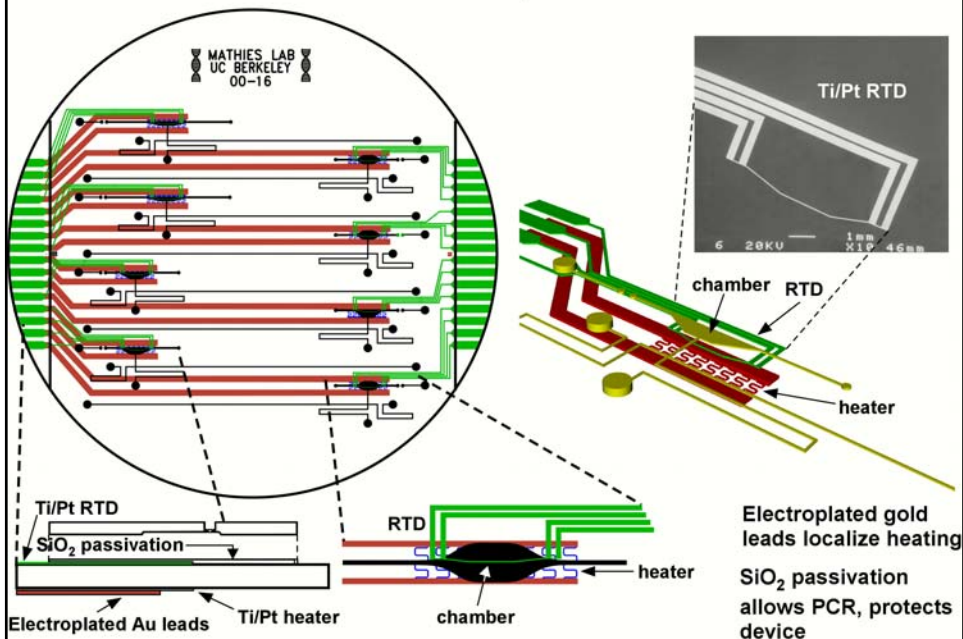
25 cycles multiplex stepdown PCR to coamplify M13 and 231 bp control



Event clustering indicative of discrete numbers of template molecules

Event frequencies fit presumed Poisson distribution (N=60)

Integrated PCR-CE Microdevice with Microfabricated Heaters and Temperature Sensors



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